

Appendix H: Summary of scientific consultation responses

1 Overview

- 1.1 The scientific consultation was carried out to gain a greater understanding of the scientific issues surrounding human-animal hybrid embryos and to determine whether or not they can be classed as live human embryos, and therefore whether their creation falls within the remit of the HFEA. Firstly there is the question whether the entities will contain a complete human genome. Secondly, it needs to be considered whether the embryos would have the potential to develop if replaced into a woman (NB: this is banned by the Human Reproductive Cloning Act 2001). Relevant stakeholders (15 scientific organisations, funding bodies and others) were asked for their views on a number of scientific questions regarding hybrids, as outlined in section 3.
- 1.2 Respondents agreed that cytoplasmic hybrid embryos contain a complete human nuclear genome i.e. 46 chromosomes. However the entities will also contain animal mitochondrial DNA (mtDNA). If human mitochondria are transferred with the nucleus then the entities will contain human mtDNA as well.
- 1.3 Respondents agreed that there was no way to test whether cytoplasmic hybrid embryos have the normal potential to develop. The presence of animal mitochondria was identified as likely to have a detrimental effect on development. There was general agreement that the embryo would be unlikely to be viable beyond early development and would be unlikely to develop if implanted in a woman. This view was supported by the Royal Society, the British Fertility Society and key researchers in the fields of mammalian embryology, developmental biology and reproductive genetics. Somatic cell nuclear replacement (SCNT) was seen as being highly complex and inefficient in the same species and respondents thought that problems were likely to be increased in experiments between different species. It was thought that the larger the evolutionary difference between species, the less likely it is that there will be normal development.
- 1.4 Views were also gathered from the HFEA's Scientific and Clinical Advances Group (SCAG), at their May 2006 and June 2007 meetings, and the Horizon Scanning Expert Panel (HHSEP), via a questionnaire in November 2006 and at their annual meeting in July 2007.
- 1.5 In summary, the broad conclusions of SCAG were:
 - Cytoplasmic hybrids would contain a full human genome and that any egg/embryo with a human genome falls within the remit of the HFE Act (e.g. parthenotes and embryos created as a result of cloning)
 - Transferred/implanted cytoplasmic hybrid embryos may not survive, but were they to do so, the human mitochondria were likely to have a replicational advantage
 - Creation of cytoplasmic hybrids could be justified for research projects due to lack of availability of human eggs but the technique should be demonstrated to be effective in animal models first

The broad conclusions of the HHSEP were:

- Cytoplasmic hybrids would contain a complete human genome, with the exception of human mitochondrial DNA
- These entities were unlikely to be viable beyond the early developmental stages

- At some stage after embryonic genome activation all proteins produced in cytoplasmic hybrids embryos (with the exception of those coded by the animal mitochondrial genes) would be human
- Cytoplasmic hybrid embryos would contain a mixture of human and animal mitochondrial DNA which would have a negative effect on their development, reducing their viability

2 Relevant stakeholders

2.1 Stakeholders who responded to the scientific consultation:

- Medical Research Council (MRC)
- Wellcome Trust
- The Royal Society
- Association of Medical Research Charities
- Motor Neurone Disease (MND) Association
- Human Genetics Alert
- Association for Clinical Embryologists (ACE)
- Royal College of Obstetrics and Gynaecologists (RCOG)
- British Fertility Society (BFS)
- Scottish Stem Cell Network (SSCN)

3 Summary of responses to the scientific consultation

Question 1: Do you think creating embryos by cell nuclear replacement (CNR) into animal eggs will be beneficial to research? Please give reasons for your answer.

- 3.1 Human Genetics Alert expressed the view that creating hybrid embryos would not be useful for research. However, this organisation is opposed to the creation of any type of embryos purely for research. They thought that due to the limited success of creating cloned human embryonic stem cell lines it may not be a useful strategy to make interspecies embryos in which there is a risk that the cytoplasmic factors and nuclear components will be mismatched. They were of the view that these entities will have mitochondria with mixed species proteins and it is likely that human proteins will not interact properly with animal proteins, thereby rapidly killing off mitochondria which produce the cell's energy.
- 3.2 The majority of organisations expressed the view that although it is unknown whether cell nuclear replacement (CNR) will prove to be a viable method for generating stem cells, all avenues of research, which may lead to greater understanding of and treatments for diseases, should be explored. They felt that although there is a wide range of views and ethical issues on this subject, on balance the creation of hybrid and chimera embryos offers important opportunities for research into a wide range of important medical conditions while not harming any existing person or human embryo, and should therefore be allowed. The technique was seen to provide a valuable experimental tool and may ultimately lead to therapeutic benefits.
- 3.3 In their response the Motor Neurone Disease Association stated that:

“The possibility of programming human embryonic stem cells genetically identical to someone affected by MND, into human motor neurones offers the potential of the most accurate model of human MND to date....There is no source of eggs from women living with MND, due to the progressive nature of the disease, and the potentially harmful effects of the IVF hormones and procedures on their MND. Thus the use of animal eggs offers an alternative method of developing human motor neurones.”

- 3.4 If animals eggs from abattoir material were used this technique would support the Royal Society's principle of the three R's. This means every effort must be made to: replace the use of live animals by non-animal alternatives; reduce the number of animals used in research to the minimum required for meaningful results; and to refine the procedures so that the degree of suffering is kept to a minimum.
- 3.5 In summary the following benefits of the technique were suggested:
- The use of animal eggs will provide the necessary large number of oocytes for this research to progress whilst avoiding the complex situation of IVF patients donating eggs to research. This will allow scientists to improve the technical efficiency of CNR so that a much smaller number of human eggs could subsequently be used to generate stem cells.
 - The basic biology of stem cells created using this technique could be investigated. This technique may provide valuable experimental models of reprogramming of gene expression, facilitating further understanding of the mechanisms of reprogramming and of the factors required to establish pluripotency.
 - This technique could be used to investigate the inheritance of mitochondrial DNA and investigate ways to reduce heteroplasmy with the aim of enhancing the reproductive success of 'older' oocytes or developing therapies for mitochondrial diseases.
 - Research using this technique may subsequently inform the development of alternative methods to derive embryonic stem cells directly from somatic cells, without the need for oocytes or early embryos.
 - This technique could provide invaluable models of cellular disease, for example, motor neurone disease, Parkinson's disease, diabetes and Alzheimer's disease and may eventually lead to the development of therapies for these diseases.

Question 2: The applications that we have received relate to a very specific aspect of 'hybrids and chimeras' (the creation of cytoplasmic hybrid embryos). Can you think of any reasons why scientists or researchers may wish to create other embryos where there is a mix of human and animal cells or DNA?

- 3.6 The general view of organisations consulted was that currently there is no reason why scientists would want to create human transgenic embryos, true hybrids or human chimera embryos.
- 3.7 It was suggested by two organisations that there is likely to be more of a case for the creation of human-human transgenic embryos for research than human-animal. One organisation referred to The Academy of Medical Sciences report on interspecies embryos which stated that researchers will at some stage have good reasons to conduct research involving the creation of human-human transgenic embryos. These techniques could facilitate the investigation of gene function in early embryogenesis or, for example, a gene could be introduced in a human embryo to increase the efficiency of the derivation of stem cells.
- 3.8 A number of the responses pointed out that the creation of transgenic animals, by introducing human genes into animal embryos has been standard scientific practice for over 20 years for investigating functions of genes and their mechanisms of regulation e.g. a number of transgenic animal models of motor neurone disease exist. Also, animal chimeras (animal embryos with human cells) are useful for a number of research purposes e.g. models of human disease, identifying signals that determine early stages of differentiation and testing developmental potential of human embryonic stem cells.

- 3.9 Therefore there is evidence that these techniques are successful in animal studies, so in theory they could be technically possible on human embryos. However, one organisation pointed out that the fact that technology for genetic modification of embryos has existed for so long without any demand for its use on human embryos suggests that it is not currently useful or practicable.
- 3.10 Although there is not currently a demand for the creation of these entities it was generally thought that it is always difficult to predict how scientific research may develop in the future.

Question 3: Can you anticipate any biological problems with embryos, or stem cells derived from embryos, created by CNR using animal oocytes that will limit their use in research?

- 3.11 The general view of organisations consulted is that it is still unknown whether this technique will prove to be a viable method of generating stem cells. Somatic nuclear reprogramming is highly complex and it has been shown to have a low success rate and give rise to abnormalities in some species models.
- 3.12 The British Fertility Society gave the view that problems are likely to arise from: mitochondrial heteroplasmy, epigenetics (incorrect remethylation of the genome) and possibly incorrect activation of the human embryonic genome in response to animal rather than human cytoplasmic factors. This view was reflected in the majority of responses received and it was generally felt that there are likely to be problems with interactions between the human derived nucleus and predominantly animal derived mitochondria e.g. improper interaction between human and animal derived proteins.
- 3.13 In their response Human Genetics Alert stated that:

“In the proposed experiments, the scientists are hoping for thousands of cross species molecular interactions, between both the mitochondria and the cytoplasm of the egg and the nuclear genes and proteins to work perfectly, in order to produce a normal cell. Different mammalian species have differences in the programmes of gene expression in early development, so it is optimistic in the extreme to expect this to work.”

- 3.14 The Scottish Stem Cell Network pointed out that stem cell lines derived from cytoplasmic embryos are unlikely to be useful models for diseases involving abnormal function of mitochondria due to the likely mixture of human and animal mitochondria.
- 3.15 The Royal Society suggested that, as it is possible to grow ES cells in culture conditions where mitochondrial function is not required and as most of the proposed research on ES cells would be conducted *in vitro*, this suggests that any problems with mitochondrial function may largely be overcome. However, *in vivo* experiments with the cells might be compromised.
- 3.16 A number of organisations also suggested that there is a risk of animal disease transmission to embryos created with animal eggs, which will mean that ES cells derived from cytoplasmic hybrid embryos are unlikely to ever be used in clinical therapies.

*Question 4: Are you aware of any data or information that would indicate that embryos created by CNR using animal eggs would not have the normal potential to develop if replaced into a woman?
NB: this is banned by the Human Reproductive Cloning Act 2001.*

- 3.17 The general view was that the question of whether cytoplasmic hybrids would have the normal potential to develop could ultimately only be answered by carrying out illegal experiments. However, there is a large amount of information from animal cloning which shows that animal embryos produced by somatic cell nuclear transfer have a very reduced potential for development, and those animals that develop are likely to be abnormal.

3.18 The Royal Society pointed out that:

“Successful implantation requires a highly co-ordinated series of cell and tissue interactions and, to date, there has been little success with animal interspecies embryo transfer. For example, mice into vole and vice versa fail at implantation because the embryo and uterine tissues do not recognise one another, whilst interspecific transfers between the more closely related sheep and goat usually implant successfully but fail in mid-gestation for immunological reasons ... Whether implantation would be affected by differential display of animal proteins on the developing embryo and the human host is unknown. There is the possibility that relevant proteins would be replaced by human proteins once transcription of nuclear genomes has begun, however, while this is very likely, details with respect to timing and extent are unknown. If implantation was to occur, but there were problems with mitochondrial replication or function it is likely that the embryo would fail at gastrulation stages.”

Question 5: Do you consider a cytoplasmic hybrid embryo to contain a complete human genome?

- 3.19 The majority of organisations are of the view that for cytoplasmic hybrid embryos to be classed as having a complete human genome they would need to contain the complete human mitochondrial, as well as nuclear, genome.
- 3.20 Cytoplasmic hybrids would contain a complete human nuclear genome but the presence of a human mitochondrial genome would depend on the number of human mitochondria transferred with the nucleus and whether they are replicated. If no human mitochondria are transferred in the process of SCNT then the cytoplasmic hybrid will be missing the 0.3% of genes which are mitochondrial.
- 3.21 One organisation gave the view that as the normal procedure for creation of cytoplasmic hybrids is to insert the entire human somatic cell into the animal egg both the nuclear and mitochondrial DNA will be included.

4 Non-respondents

- 4.1 Responses were received from 10 of the 15 organisations the scientific questions were posed to. Three of these organisations did not specifically answer the questions in their responses.
- 4.2 Out of the 5 non-respondents, one funding body did not respond because they did not expect to fund the creation of human-animal embryos as the research is unlikely to fall within their remit. One organisation thought that it would not be appropriate to respond as individual scientists within the organisation would be providing the HFEA with information.

5 Scientific and Clinical Advances Group (SCAG)

5.1 Members of SCAG

- Professor Neva Haites
Professor in Medical Genetics, University of Aberdeen
- Professor Chris Barratt
Scientific Director, Birmingham Women’s Health Care
- Mr Roger Neuberg
Consultant Gynaecologist, Leicester Royal Infirmary
- Dr Maybeth Jamieson
Consultant Embryologist, Glasgow Royal Infirmary
- Professor Peter Braude

Professor of Obstetrics & Gynaecology, King's College London

- The Right Reverend Lord Harries of Pentregarth
- Ms Clare Brown
Chief Executive, Infertility Network UK
- Professor Lorraine Young
School of Human Development, University of Nottingham
- Miss Melanie Davies
Consultant Gynaecologist, University College London Hospital
- Professor Richard Gardner
Department of Zoology, University of Oxford
- Dr Daniel Brison
Scientific Director, Department of Reproductive Medicine, University of Manchester
- Professor David Barlow
Executive Dean of Medicine, University of Glasgow
- Dr Robin Lovell-Badge
Division of Stem Cell Biology and Developmental Genetics, The National Institute for Medical Research

5.2 In May 2006 SCAG was asked to give a view on whether an interspecies cytoplasmic hybrid embryo would be human.

Responses

5.3 Members were of the view that particular consideration needs to be given to the role of mitochondria, as it is unknown what the proportion of contribution from human and animal mitochondria will be in these hybrid embryos. Members were of the view that if hybrid embryos were transferred/implanted they may not survive, but if they do then human mitochondria are likely to have a replicational advantage as they are more compatible with the genome. The group expected that if cell lines were derived from these embryos and cultured, then it is likely that the human mitochondria will dominate over the animal mitochondria, although this has not been proven.

5.4 One issue raised was whether the hybrid embryo would be human from the two cell stage, or only become gradually human after a number of days development. One SCAG member was of the opinion that for the first 5 or 6 days of development the entity would initially be predominantly animal because it would contain animal proteins (proteins coded for by animal DNA). It would then become gradually humanised, becoming predominantly human by 8 or 9 days of development.

5.5 The general opinion of the group was that interspecies cytoplasmic hybrid embryos should be classed as human.

5.6 In June 2007 SCAG were asked for their views on the questions posed to stakeholders, as outlined in section 1.

Responses

5.7 Members were of the view that the creation of hybrids is necessary for research projects due to lack of availability of human eggs. These research projects could include investigating the interaction of mitochondrial and nuclear DNA in order to study mitochondrial diseases. These hybrids could also be used for many of the same research purposes that have been proposed for SCNT using human oocytes.

5.8 Members agreed that all avenues of research should be explored. One member noted that cell nuclear transfer is poorly understood and that research on nuclei and cytoplasm interactions need to be carried out from human to animal, animal to human and animal to

animal. Another member suggested that animal-animal models should be carried out first. Literature on the use of animal-animal hybrids for the conservation of rare species was highlighted as a potentially useful resource. The Group were of the view that creating cytoplasmic hybrid embryos would involve less genetic manipulation than other models, such as reprogramming fibroblasts.

- 5.9 The Group were of the opinion that there is no scientific case for true interspecies hybrids.
- 5.10 Members thought it was impossible to tell if embryos created in this way would have the normal potential to develop if replaced in a woman, and there was no way of testing it. One member was of the opinion that mitochondrial function would be severely compromised in a significant proportion of cells around the gastrulation stage and that embryo development beyond this would be very abnormal. It was noted that although the embryo may fail, this would not prevent embryonic stem cell derivation, because this only requires one or a few cells and because they have little requirement for mitochondrial function. One member pointed out that the HFEA already regulates research on types of embryo that have little or no normal potential to develop if they are replaced in a woman, e.g. embryos carrying chromosomal or severe genetic abnormalities and parthenogenetic embryos.
- 5.11 The group thought that the mitochondrial element of the genome had to be taken seriously and that epigenetics were important. One member was of the opinion that a cytoplasmic hybrid would not contain a complete human genome, but would contain 46 chromosomes. Another member thought that it would contain a complete human genome because it will contain both nuclear and mitochondrial genomes from the human donor somatic cell. It was suggested that some human mitochondria would have to be transferred with the nuclear DNA. The group thought that at different stages different proportions of the human genome would be present and there was some concern that using eggs from a different species would change the gene expression because the nucleus will be surrounded by proteins from the host egg.

6 HFEA Horizon Scanning Expert Panel (HHSEP)

6.1 Members of HHSEP who responded:

- Professor David Edgar
School of Biomedical Sciences, University of Liverpool (research interests - development biology, human embryology)
- Dr Maureen Wood
Research Embryologist, Assisted Reproduction Unit, University of Aberdeen
- Professor Peter Andrews
Department of Biomedical Sciences and the Centre for Stem Cell Biology, University of Sheffield
- Professor Alan Trounson
Monash Institute of Reproduction and Development, Monash University, Australia
- Professor Henry Leese
Department of Biology, University of York (research interests - biochemistry and physiology of early mammalian embryos)

6.2 The advice of the HFEA's Horizon Scanning Expert Panel was initially sought on the issues of hybrids, by sending out the following questionnaire, in November 2006:

- 1) *Would any entity created by activating a human somatic cell nucleus within an enucleated animal (e.g. cow or rabbit) oocyte:*
- a) be viable, or, at least, possibly viable?*
 - b) contain a complete human genome?*
 - c) be a human embryo?*

d) ever have the potential to develop and result in a live birth, if implanted? (N.B. the HFE Act 1990 prohibits this)

2) Given that the proteins present would be predominantly animal, would the entity created be human from the moment of activation? If not, at what stage, in your opinion, would the entity become human? How long would the animal proteins be present?

3) What would be the significance and likely effect of the presence of animal mitochondrial DNA on any such entity's development?

Also, at their annual meeting on 2nd July 2007 the Panel discussed whether the creation of hybrids and chimeras would be beneficial for research.

Responses

- 6.3 Panel members agreed that the entity would contain a complete human genome with the exception of human mitochondrial DNA (mtDNA). One panel member was of the opinion that the entity may contain human mitochondrial DNA, as well as nuclear DNA, as mitochondria transferred with the donor nucleus may be preferentially replicated. It was pointed out that the mitochondrial genome is very small and only encodes a few mitochondrial proteins relating to oxidative phosphorylation.
- 6.4 There were mixed views on the potential of these entities to develop, but it was generally thought that they were unlikely to be viable beyond the early developmental stages. One member suggested that data from animal models suggests that large species differences make it unlikely. It was noted that some cross-species cloning has produced offspring but this tends to be between closely-related species. The interspecies problems of mitochondrial and nuclear compatibility were raised. One member thought that entity would be capable of normal developmental behaviour, at least in the initial stages, but would expect increasing problems as development proceeds. Another member stated that the entity cannot develop to term and that there is no evidence for this at all.
- 6.5 The general view of Panel members was that at some stage after embryonic genome activation all proteins produced (with the exception of those coded by the animal mitochondrial genes) would be human. One member thought that most proteins would be human within a few rounds of cell replication and certainly by the time of implantation. It was noted that in humans, embryonic genome activation does not happen until between the four and eight cell stage. Therefore, until this stage, the embryo is relying on proteins and genetic messages that were present in the oocyte (i.e. from the animal) and that the entity may not be regarded as 'fully human' during this early period. One member thought that any stem cells formed would be almost entirely human.
- 6.6 Members who felt able to answer question 3 were mostly of the view that the mixture of human and animal mitochondrial DNA would have a negative effect on the development of this entity, reducing its viability. These entities may be more viable if the animal mitochondrial DNA is eliminated, although it was noted that some papers have argued that somatic mtDNA hinders embryonic development. However one member thought that the persistence of a few animal mitochondrial genes would not have much significance for the behaviour of the resulting entity. One member thought that there is a high risk of epigenetic change and disruption of development. Members felt that the work on animal cybrids (the fusion of an enucleated somatic cell with a somatic cell) would be worth reviewing. It was noted that experiments creating human and primate cybrid cell lines resulted in slower growth and respiratory rates. When the evolutionary distance was too diverse, effective cellular function could not be sustained.
- 6.7 At their annual meeting on 2nd July 2007 the Panel discussed whether the creation of hybrids and chimeras would be beneficial for research. The Panel members expressed

mixed views as to whether creating interspecies cytoplasmic hybrid embryos would be beneficial for research. One panel member was of the view that the limited work that has been carried out on animal-animal interspecies cytoplasmic hybrid embryos has shown that stem cell lines derived from these entities show slow cell replication, that there is no connection between the mitochondrial and nuclear components of the embryo and the method is currently inefficient. It was also suggested that there are a number of other sources of embryos and methods to create stem cell lines before the creation of interspecies hybrid embryos should be considered. These sources/methods include:

- Mitotically arrested zygotes
- Triploid embryos
- Tri or mono pronuclear eggs
- Reprogramming somatic cells

Another panel member was of the view that scientists in the UK should be allowed to create interspecies cytoplasmic hybrid embryos in order to demonstrate whether or not it is possible to repeat the results of the groups who have reported the creation of human-rabbit and human-cow entities. This panel member felt that every avenue of research should be explored and that the creation of hybrids should be permitted as they will never be transferred to a woman and allowed to implant, as there is regulation in place to prevent this happening.